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Effect of the mother tree age and acorn weight in the regenerative characteristics of *Quercus faginea*

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Abstract

The establishment of oak trees is often a slow and difficult process. Hence, it is necessary to determine the characteristics that can lead to improving their regeneration. In this genus, seed size is highly variable both at the interspecific and intraspecific levels, and the effects of intrapopulation variability are not well understood, being even less so for *Quercus faginea*. In this study, the effects of the age of the mother tree, seed weight and the interaction between these two factors on seed germination, emergence and growth (biomass) were analysed. For this purpose, 16 trees —8 young and 8 old— were selected with the intent to cover the entire range of acorn weights produced in this population. Among the main results, it should be noted that: (1) in older trees, it is easier to find larger acorns; (2) the percentage and the speed of germination of the acorns of young trees is greater than that of old trees; (3) the percentage and the speed of seedling emergence of young trees is greater than that of old trees; and (4) cotyledon weight is the variable that most influences biomass, quite often in a positive way. Therefore, maintaining intrapopulation variability seems to be an approach that most favours the persistence of these populations.

Keywords: Emergency, Germination, Mother tree age, *Quercus faginea,* Reforestry, Seed size, Seedling biomass

Introduction

The *Quercus* genus is able to adapt to most of the environmental conditions of the Iberian Peninsula, owing to the variety of leaf lifespan strategies of the deciduous, perennial and marcescent species (Blanco et al. 1996). In addition, anthropogenic activities are the main cause of the configuration of the landscape of the Mediterranean basin due to livestock, agricultural and forestry practices (Blanco et al. 1996; Bergmeier et al. 2010). Consequently, the real landscape is comprised of a mosaic of forests interspersed among agricultural crops, grasslands, shrubs, open woodlands and forests (Blanco et al. 1996), where the areas occupied by *Quercus* species have greatly been reduced. Although the current distribution of *Quercus* is still wide, it only represents a very small sample of its once original distribution (San Miguel-Ayanz 1985). Moreover, the reforestation of Mediterranean areas with *Quercus* species, which is a slow growing genus (Villar et al. 2008; Costa et al. 2017) in an environment fragmented by human activities, is not an easy task.

In this study, we will specifically focus on the species *Quercus faginea* Lam. *Q. faginea*, which mainly has a western circumboreal distribution (Ceballos and Ruiz de la Torre 1971). Its chorology is markedly Ibero–North African, with the exception that this species can be found towards the south of France and on the island of Mallorca (Blanco et al. 1996). *Q. faginea* has a wide ecological valence and grows at altitudes of 500–1500 m in all types of soils, including those with small or large amounts of lime. Some of its races hold up well in continental climates, with strong contrasts between temperature and humidity. These characteristics probably make it the Iberian oak with the greatest thermal amplitude. However, *Q. faginea* is the most affected species for deforestation and the associated soil degradation, and the historic preference that humans had by *Quercus ilex* (Blanco et al. 1996).

In addition to anthropogenic activities, other external factors affect the early establishment of *Quercus* such as soil dryness, predation, phytopathogens and rainfall. (Pérez-Ramos et al. 2010; Costa et al. 2017). Also, animals use the acorns of *Quercus* species as a food source. These animals include small invertebrates (e.g. Coleoptera or Lepidoptera larvae), large vertebrates (red and roe deer, cattle, wild boars, etc.) and other small species (rodents, birds, lagomorphs) (Pérez-Ramos et al. 2008; Del Arco et al. 2018). In addition, many of these species can have periodic high population densities that cause the massive consumption of acorns (Del Arco et al. 2018).

These external factors, however, do not act alone, and some intrinsic factors can affect the success of the establishment of seedlings. One of these factors is acorn weight, since they make up the reserves that are available in the first stages of life (Khan and Shankar 2001; Gómez 2004; Quero et al. 2007; Fahrettin 2010). The weight of the seeds of the *Quercus* species is highly variable (Ramírez-Valiente et al. 2009), especially at the intraspecific level, whether individuals belong to the same or different populations (Gómez 2004). Another interesting intrinsic factor is the regenerative characteristics of the mother tree, of which very little is known. Some studies have reported that intrapopulation variability among *Quercus* trees can change the relationship between seed weight and the traits related to seedling survival (González-Rodríguez et al. 2011). In one study, differences in regenerative characteristics (germination, emergence and growth) were observed when the age of the *Q. ilex* mother trees was taking into account (Fernández-Santos et al. 2013). No other publications on the germination of *Quercus* species have been found which consider mother tree age. However, the effect of the age of trees on the germination of their seeds has been analysed in other species such as *Sorbus torminalis* (Espahbodi et al. 2007) and more recently in *Sapindus mukorossi* (Bisht et al. 2016), *Acacia melanoxylon* (Cruz et al. 2017) and *Pinus pinaster* (Cruz et al. 2019). Therefore, including “mother tree age” in studies on the regeneration of *Quercus* species may help to better understand intrapopulation variability.

In this study, the intrapopulation variability of the regenerative characteristics of *Q. faginea* has been examined by considering two varying factors: mother tree age and acorn weight. To do so, in this work, we analysed: (1) variability in the weight of the acorns of the trees of most contrasted sizes in a population; (2) the percentage and speed of the germination of acorns (days elapsed until germination); (3) the percentage and speed of seedling emergence (days elapsed until the seedlings appear); and (4) seedling growth (aboveground biomass, belowground biomass and total biomass per plant).

Materials and methods

Study area

The study area is located in the north subdivision of the central plateau of the Iberian Peninsula (41°07'N, 5°47'W; 790–820 m a.s.l.; Fig. 1). Eutric and humic cambisols are the dominant soils (Dorronsoro 1992). Mean annual precipitation varies between 400 and 450 mm with a typical Mediterranean period of low precipitation during July and August. The mean annual temperature is between 11 and 12 °C, with mean temperatures between 3 and 4 °C and 20–21 °C during the coldest and the warmest months, respectively. The study area is characterized by a variety of land covers, with open and closed woodlands (dominated by *Q. ilex* subsp. *rotundifolia*, *Q. suber*, *Q. faginea* and a minor presence of *Q. pyrenaica*), plantations of *Pinus pinaster*, shrublands dominated by *Cistus* and *Cytisus* species, old fields and abandoned vineyards.

Selection of the mother trees and seed collection

Sixteen mother trees were selected within the same population (Table 1): eight fully mature trees, with a diameter at breast height (DBH) of ≥ 50 cm (old trees), and eight young trees with a DBH of < 20 cm. The trees were selected in such a way as to cover the widest range possible of acorn sizes existing within the population for each age category. First, 15 random points were deployed, and the nearest tree of each diameter class was selected per point. Only trees with healthy acorns and enough acorn production for the experiment were considered. Secondly, of every 15 trees per diameter class, eight trees were retained and seven were discarded using a stratified random sampling approach covering the widest range of acorn sizes. Lastly, we walked through the woodland looking for trees with more extreme acorn sizes, and the trees Y8, O1 and O2 were selected for the experiment. The acorns were collected in October, which coincided with optimal maturation (López-González 2001). The seeds were collected manually by shaking the branches with a stick.

Increment cores from all trees were extracted using Pressler increment borers (Häglof, Sweden) at 0.2–0.3 m above ground level to obtain the most accurate age of each tree. Increment cores were mounted and sanded following the procedure established by Stokes and Smiley (1968), and the annual rings were counted using a stereomicroscope (SMZ800, Nikon, Japan).

Experimental design

The acorns were stored at a temperature between 2 and 4 °C until used to carry out the experiments. First, the acorns were visually inspected and then subjected to a flotation test, and the damaged seeds were discarded. The remaining acorns were allowed to air-dry for a few days, and then, 50 acorns were randomly selected from each mother tree. A total of 800 acorns (50 acorns × 8 mothers × 2 ages) were obtained, and their fresh weight was measured (Analytical balance Sartorius CP 124S—Precision 0.1 mg). Twenty acorns originating from each tree were used to obtain the regression lines in order to estimate the dry weight of the cotyledons of the acorns sown. To do this, the acorns were oven-dried at 70 °C for a minimum of 24 h and subsequently weighed to obtain the dry mass of the acorn. The pericarp was removed from the cotyledons and reweighed to obtain the dry weight of each cotyledon. Linear regression equations for acorn fresh mass and cotyledons dry mass were developed for each mother tree, which permitted the cotyledon dry mass to be estimated from the acorn fresh mass (R_2 ranging between 0.72 and 0.96) (Appendix, see Supplemental Data with the online version of this article).

The remaining 30 acorns of each parental tree were sown, and the germination, emergence and growth of each were analysed. After weighting each fresh acorn, they were individually sown in pots under laboratory conditions. At the beginning of the experiment, the acorns were sown on the surface of the soil to be able to record the time of germination. Then, the acorns were buried horizontally into the soil at a depth of 1–2 cm for observing seedling emergence. Acorn layout was carried out following a model of random distribution in pots (type S.1 35, 235 cm_3, 16 cm deep × 5 cm wide) filled with a 1:1 mixture of soil, coming from the area where the seeds were collected, and peat. The plants were watered until saturation twice a week with distilled water to ensure that water was not a limiting factor in their growth. The plants were grown under laboratory conditions; the mean temperature was 19.7 ± 6.3 °C during the course of the experiment that lasted for 6 months.

Data collection

From December 2014 until May 2015, the data collection was done. Twice a week, germination and emergence dates were registered. When the seedlings showed the first group of 4–5 leaves completely unfurled (Green and Juniper 2004; González-Rodríguez et al. 2011), which correspond to the moment in which they stopped relying exclusively on cotyledon reserves (Green and Juniper 2004; González-Rodríguez et al. 2011), the seedlings were harvested, and each plant was washed with water and fractioned in different parts, leaves, stem, fine roots and thick roots. All parts of the seedlings were dried at 70° for 24 h, and the aerial and subterranean fractions were weighted (Analytical balance Sartorius CP 124S—Precision 0.1 mg).

Statistical analysis

To mean values, Student's *t* test or a one-way ANOVA was used, according to the number of groups to be compared, after checking normality and homoscedasticity. To analyse the elapsed time until germination or emergence and biomass variables, linear models (LM) were used, establishing as a dependent variable number days until germination, number days until emergency or biomass, as an independent variable fixed factor "Mother Tree Age" (young or old) and the quantitative variable "Dry weight of the Cotyledon", also

taking into account the interaction between both factors. The IBM SPSS Statistics 19 program was used to carry out the statistical analysis.

Results

Tree ages

The young trees had an average age of 29.4 ± 1.6 years (mean ± SE, Table 1). However, only minimum ages were estimated for the old trees as the trees had rotten centres. The average minimum age was estimated to be 210 ± 17.7 years (mean ± SE, Table 1).

Cotyledon weights

There was a wide range of acorn weights among the trees within the population for both the young group (0.61–4.39 g dry weigh) and the old group (0.80–4.40 g dry weigh) (Table 1), although it was more difficult to find young trees with large acorns and old trees with small acorns. The values obtained for themean cotyledon dry weights for each selected tree were between 0.44 and 3.41 g for the young trees and between 0.64 and 3.51 g for old trees (Table 1). It was made a comparison among the mean values of the extrapolate data for the dry cotyledon weight of sown acorns, and statistically significant differences were detected for most of the mother trees (Table 1) not being able to differentiate clear categories of sizes for the two age groups; because of that, cotyledon dry weight will be taken as continuous variable for the following analyses.

Germination and emergence

The germination and emergence (%) values obtained for each tree are shown in Table 1. When germination values were analysed, statistically significant differences were detected between young and old trees (*t*: 2.798, gl: 14, *p* = 0.014), being the mean value of young trees (51.7%) significantly higher than the old ones (34.6%) (Fig. 2a).

In addition, the percentages of seedling emergence obtained for both young and old trees also showed statistically significant differences (*t*: 2.717, gl: 14, *p* = 0.017), where the mean value for young trees (47.1%) was significantly higher than the value corresponding to the old trees (30.8%) (Fig. 2b).

The analysis of the time elapsing between sowing and germination (Fig. 3a) detected statistically significant differences with respect to mother tree age (*F*: 3.918, *p* = 0.049).

However, this was not the case for the cotyledon dry weight (*F*: 0.157, *p* = 0.693) or the interaction between these two factors (*F*: 0.615, *p* = 0.434) (Table 2). As can be seen in Fig. 3a, the acorns from young trees germinated before (mean value 24.4 days) those coming from old trees (mean value 46.9 days), and it did not depend on the dry cotyledon weight.

The analysis of the time elapsing until seedling emergence (Fig. 3b) detected that mother tree age may be important (*F*: 3.571, *p* = 0.060), since significant differences were detected (Table 2). This again was not the case for cotyledon dry weight (*F*: 0.079, *p* = 0.779) or the interaction between both factors (*F*: 0.242, *p* = 0.623). Seedling emergence from acorns collected from old trees took longer (mean value = 70.4 days) than those coming from the young trees (mean value = 59.8 days) (Fig. 3b).

Biomass

With respect to the variables associated with biomass, cotyledon dry weight appeared to have a significant influence. However, in contrast, mother tree age and the interaction between the two factors did not (Table 2). Also, cotyledon dry weight appeared to have a positive influence on biomass (Fig. 3): total biomass (*F*: 108.915, $p = 0.001$; Fig. 4a); aboveground biomass (*F*: 46.321 $p = 0.001$; Fig. 4b); and belowground biomass (*F*: 123.769, $p = 0.001$; Fig. 4c). Biomass increased as the cotyledon weight increased. On the other hand, a negative influence of cotyledon dry weight was detected over some variables (Fig. 5): aboveground biomass/total biomass (*F*: 5.166, $p = 0.025$; Fig. 5a) and aboveground biomass/belowground biomass (*F*: 5.557, $p = 0.020$; Fig. 5c) increased with the decrease in the cotyledon. Although belowground biomass/total biomass (*F*: 5.166, $p = 0.025$; Fig. 5b) increased with the increase in cotyledon weight, the acorns weighing less gave rise to seedlings with more aboveground with respect to the belowground biomass thanthe acorns of higher weights.

Discussion

Effect of intrapopulation variability

In this work, the average weights of the acorns collected from different trees are highly variable. The range of the average acorn weight per tree (the average values of acorn dry mass per tree 0.61 and 4.40 and between 0.44 and 3.51 for seed dry mass) is wider than that registered for other populations of *Q. faginea* [with a seed dry mass ranging between 0.76 and 3.32 in González-Rodríguez et al. (2011) and 0.87–3.08 in González-Rodríguez et al. (2012)]. However, these weights are quite similar to those recorded for other *Quercus* populations (Leiva and Fernández-Alés 1998; González-Rodríguez et al. 2011, 2012; Fernández-Santos et al. 2013). Moreover, a high level of variability was found among the seed dry weights calculated for both young and old trees (young 0.44–3.41 g, old 0.64–3.51 g). In addition, it was difficult to find old mother trees with small acorns and young mother trees with large acorns, which also occurred in a population of *Q. ilex* located in the same study region (Fernández-Santos et al. 2013). For other species, such as *Pinus nigra*, tree age did not seem to have a significant influence on seed size (Alejano et al. 2019).

Germination

In this study on *Q. faginea*, the age of the mother tree had a clear influence on germination, independent of the dry weight of the cotyledon. This influence is statistically significant and is observed for both the germination rate and for the time elapsing until acorn germinates. The seeds produced by young mother trees are more likely to germinate, and more quickly, than those produced by old mother trees. In terms of the germination rate, the acorns of the young mother trees germinate in greater proportion than those produced by the old mother trees (mean 51% and 35%, respectively). In the few existing studies on *Quercus* species, this effect has not detected. In Fernández-Santos et al. (2013), no significant differences in the percentage of germinated seeds have been found between the acorns produced by young and old trees for *Q. ilex*. For other species, such as *Pinus nigra* (Tíscar-Oliver 2002; Alejano et al. 2019) and *Acacia melanoxylon* (Cruz et al. 2017), the age of the mother tree did not seem to have an influence on the final germination values. However, in a study on *Sorbus torminalis*, Espahbodi et al. (2007) detected differences in the germination rate according to the age of the mothers. The highest germination rate was obtained for seeds produced by middle-aged trees, and similar to our study, no correlation is found between the weight of the seeds and the percentage of germination. Regarding the effect of the weight of the acorn on germination, in this study, no significant influence was detected. The same finding has also been reported by Fernández-Santos et al. (2013) for *Q. ilex,* although in several other studies, higher germination rates for large seeds of *Q. ilex* (Gómez 2004) and *Q. suber* (Quero et al. 2009) were observed, and also in negative humidity conditions for *Q. suber* (Urbieta et al. 2008). Furthermore, when assessing the time it takes for acorns to germinate, in this study, it can be seen that acorns from young mother trees take approximately half the number of days to germinate on average than those from old mother trees (average 24 and 50 days, respectively). Seed weight also has no effect on germination. Previously, in Fernández-Santos et al. (2013), it was reported that mother tree age and acorn have a joint influence on the rate of germination in *Q. ilex*, where the small acorns of young trees germinate the fastest. Tíscar-Oliver (2002) also detected for *Pinus nigra* subsp. *salzmannii* that the seeds of old trees take longer to germinate, but the seeds are smaller, and Cruz et al. (2017) showed that *A. melanoxylon* mother plant age influences the time in which the first germinations occur after fire. Other studies also show that the germination time of the seeds varied with the provenances but not with seed mass in *P. pinaster*, although mother age was not considered as a potential influencing factor (Calvo et al. 2016).

Emergence

Seedling emergence of *Q. faginea* is clearly influenced by mother tree age, independent of cotyledon dry weight. This influence is statistically significant for the emergency percentage and probably significant for the emergency speed. The seedlings produced by young mother trees are more likely to emerge and more quickly than those produced by old trees. Again, acorn weight seemed not to have an effect. Regarding the rate of seedling emergence, it can be said that the acorns of young mother trees emerge in greater numbers than those produced by the old mother trees (mean 47% and 33%, respectively). This effect has not been previously detected. Fernández-Santos et al. (2013) are unable to draw clear conclusions about the effect of the mother age on seedling emergence in *Q. ilex*. González-Rodríguez et al. (2011), who do not consider mother tree age in their study on *Q. faginea*, do not find any interaction between these two factors. In this study, acorn weight has not appeared to be an influencing factor on seedling emergence as has been shown in previous studies on *Q. ilex* (Gómez 2004) or *Q. canariensis* (Urbieta et al. 2008). In contrast, Quero et al. (2009) reported a seed weight has a positive effect on seedling emergence in *Q. suber* and *Q. pyrenaica*. In Urbieta et al. (2008), it was detected that the largest seeds of *Q. suber* and *Q. pyrenaica* were more likely to emerge. For other species, Castro (1999) observes a relationship between the seed weight and the seedling emergence of *Pinus sylvestris*. When assessing the time of emergence, mother tree age does have an influence, where the acorns of young trees shorten the time of emergence by 16% with respect to the acorns of adult trees (average 59 and 70.4 days, respectively). González-Rodríguez et al. (2011) found the producing mother did not have an effect on *Q. faginea* and age was not considered. However, an effect on *Q. suber* was detected, where the speed of emergence was influenced by the acorn weight. With respect to the effect of acorn weight in this study, no influence on the time of emergence has been detected. In Urbierta et al. (2008) and Quero et al. (2009), it is confirmed that for *Q. pyrenaica*, there is a positive relationship between the time of emergence and seed weight; that is, the larger seeds took longer to emerge. In Tíscar and Lucas (2010), a negative correlation involving seeds weight is found for *Pinus nigra* subsp. *salzmannii*, depending on the type of substrate and the mother plant. Therefore, there is no consensus regarding the effect of the seed weight on the time of seedling emergence.

This study shows that acorns from young mother trees have higher speeds of germination and emergence than those produced by old mother trees in *Q. faginea*. The main advantage is that earlier growth equates to greater plant development at the time of facing environmental stresses, especially in climates with great intra- and interannual variation like the Mediterranean. So, the species of *Quercus*, which do not have dormancy and have transient soil seed banks, have shown different germination times related to the intensity of water stress during the summer (Reyes and Casal 2006). For example, acorns of *Q. ilex* showed germination times shorter than *Q. pyrenaica* and *Q. robur*, which allows an earlier seedling development in *Q. ilex* and to cope better with the typical summer drought of the Mediterranean climate (Reyes and Casal 2006).

Biomass

The analyses carried out to evaluate seedling growth indicate there is a clear significant relationship between the weight of the acorns of *Q. faginea* and the biomass of their seedlings, a relationship that is not affected by the mother tree age. A positive influence of the dry weight of the cotyledon is observed in the total biomass, belowground and aboveground biomass, which supports the hypothesis of the "seedling size effect". This indicates that larger seeds produce larger seedlings (Westoby et al. 1996), which may lead to certain adaptive advantages in the establishment of seedlings (Hendrix et al. 1991; Eriksson 1999; Chacón and Bustamante 2001; Khan 2004; Castro et al. 2008; González-Rodríguez et al. 2011). This had already been detected in other populations of *Quercus*. The work of González-Rodríguez et al. (2011) supports this hypothesis for *Q. faginea, Q. ilex, Q. suber and Q. pyrenaica*. The authors also find significant interaction between the producing mother and seed weight, depending on the species and the phase of growth. But similar to our study, the mother has no effect on the seed growth of *Q. faginea*. The effect of acorn weight has been verified for *Q. ilex* and *Q. pyrenaica* under different light conditions. This relationship is more evident under lower light intensities where the seedlings depend more on cotyledon reserves (Quero et al.

2007). For *Pinus sylvestris*, Castro (1999) suggests that this positive relationship is indirectly related to other characteristics associated with seed weight such as the genetic variability of the mother in the same population.

In this study, cotyledon weight only begins to have an influence during the growing phase and not prior to this. But some studies have observed that the most successful plants (understanding this as the combination of emergence and survival) originate from larger acorns from different species of *Quercus* (*Q. faginea, Q. ilex, Q. suber, Q. pyrenaica*) (González-Rodríguez et al. 2011), which have been sown directly into soil. It has been found that in general, plants from larger seeds have higher germination and emergence percentages and are more likely to survive, especially if the environmental conditions are adverse for *Q. ilex* (Gómez 2004) and *Q. suber, Q Canariensis and Q. pyrenaica* (Urbieta et al. 2008). In recent years, some studies have been carried out that support the idea that the biomass of a plant at a given time depends not only on its relative growth rate (RGR) but also on the initial biomass, which is determined during the first stages by the weight of the seed (Villar et al. 2008). Also, for some *Quercus* species, the size of the acorn may influence the biomass accumulation of the seedlings for 7 months or more (Quero et al. 2008). However, in the field, it could be that this effect is not as remarkable as, after dispersion, a large proportion of *Quercus* seeds are consumed by different predators (Santos and Tellería 1997; Del Arco et al. 2018) and smaller seeds tend to have a lower probability of depredation (Pérez-Ramos et al. 2008). Nevertheless, although larger seeds are more likely to be predated (Gómez 2004), it could occur that during the years of mass production (mast seeding), predation satiation (Xiaogai et al. 2010) would allow larger acorns to produce trees with higher aerial and underground biomass with a greater probability of survival (González-Rodríguez et al. 2011). Therefore, abundant crops or mast years probably contribute the most to the natural regeneration of *Q. faginea,* as has been suggested for *Pinus nigra* (Tíscar-Oliver 2002; Tíscar and Lucas 2010).

On the other hand, in this study, a negative influence of the weight of the cotyledon has been detected for aboveground biomass/total biomass and aboveground biomass/belowground biomass ratios, which indicates that smaller acorns invested more in the aerial part. Probably, this relationship is due to the fact that by having fewer reserves in the seed, there is a greater initial dependence on the photosynthetic tissues in these seedlings with respect to those that come from larger seeds (Poorter and Rose 2005). These results are consistent with the fact that seedlings tend to maximize growth in the initial stages in order to reach the deep soil layers (Escudero and Mediavilla 2003; Mediavilla and Escudero 2004) and to deal with stress water during the first summer, which is one of the most limiting factors in the successful establishment of the *Quercus* genus in Mediterranean environments (Valladares et al. 2008).

The results of this study show that the acorns from young mother trees have a clear advantage over those produced by old mother trees with regard to germination and emergence. Our findings suggest there may have been a change in selection pressures during the last decades towards earlier germination and emergency. The main advantage is that earlier growth equates to greater plant development at the time of facing water stress typical of summer. If this is correct it could explain why young trees produce acorns with higher speeds of germination and emergence, since what they would do is express a characteristic that gave them an adaptive advantage in their establishment. Variations in regenerative characteristics have also been observed in different age groups in response to changes selective pressures in other tree species, such as *Pinus pinaster* (Cruz et al. 2019). However, the differences detected in this study according to the age of *Q. faginea* trees could also be due to the fact that there are changing reproductive alternatives during the tree's lifetime, as Tíscar and Lucas (2010) suggest for *Pinus nigra*.

Conclusions

Taking into account the aforementioned, it can be concluded that within a population of *Quercus faginea*, both mother tree age and acorn weight are factors to be taken into account if successful establishment is to be favoured (germination, emergence and growth). On one hand, the presence of young trees would favour success during the germination and emergence phases, since these trees produce acorns that germinate and emerge more and faster. On the other hand, the presence of old trees in the populations would favour the presence of larger acorns, and these acorns would tend to produce larger seedlings. Both factors may be important in the natural regeneration of *Quercus* populations and should be taken into account when carrying out reforestation programs.

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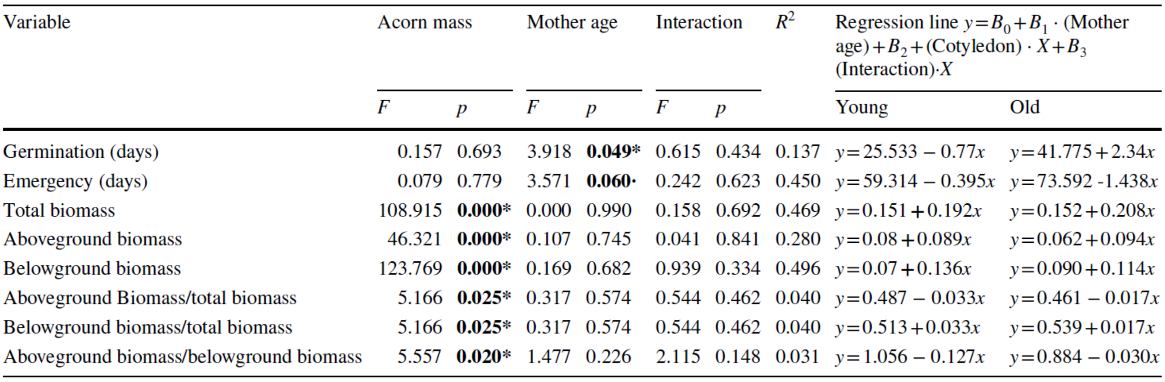
Table 1. Values obtained for each tree, young (Y) and old (O) trees selected for the study.

Tree	Diameter (cm)	Growth rings	Acorn weight	Cotyledon weight (g)	Germination (%)	Emergence (%)
Y1	11.5	26	0.61±0.15	0.44±0.14a	46.7	40.0
Y2	15.8	34	0.78±0.02	0.61±0.02a	43.3	43.3
Y3	17.1	38	1.06±0.06	0.83±0.04a	66.7	50.0
Y4	12.1	27	1.84±0.07	1.44±0.05b	50.0	46.7
Y5	10.2	24	1.85±0.05	1.45±0.04b	70.0	70.0
Y6	14.3	29	2.50±0.23	1.92±0.18bc	66.7	63.4
Y7	12.4	30	2.60±0.14	2.00±0.10c	36.7	33.3
Y8	13.7	27	4.39±0.11	3.41±0.08dg	33.3	30.0
O1	69.0		0.80±0.03	0.64±0.02a	43.3	40.0
O2	63.5	197*	0.93±0.03	0.70±0.02a	33.3	30.0
O3	66.9	110*	2.46±0.06	1.93±0.04c	53.3	50.0
O4	63.2	219*	3.43±0.08	2.64±0.05e	26.7	26.7
O5	73.9	239*	3.63±0.15	2.80±0.11ef	33.3	26.7
O6	58.7	243*	3.79±0.09	3.09±0.08df	23.3	20.0
O7	61.8	238*	4.30±0.09	3.43±0.07dg	30.0	23.3
O8	50.3	225*	4.40±0.10	3.51±0.08g	33.3	30.0

Acorn and cotyledon dry weights (g) (mean ± standard error, *n* = 30) and the results of the contrast of means comparisons; different letters indicate significant differences ($p < 0.05$) with the Games–Howell test

*The asterisks indicate the minimum ages of the trees. Due to the state of the putrefaction of the trees, the sample did not reach the centre

Table 2. Results of the contrasts made for the different variables and their relations as a function of the age of the mother tree factor and cotyledon dry weight and the interaction between both factors.



Variable	Acorn mass		Mother age		Interaction		R^2	Regression line $y=B_0+B_1\cdot$(Mother age)$+B_2+$(Cotyledon)$\cdot X+B_3$ (Interaction)$\cdot X$	
	F	*p*	*F*	*p*	*F*	*p*		Young	Old
Germination (days)	0.157	0.693	3.918	**0.049***	0.615	0.434	0.137	$y=25.533-0.77x$	$y=41.775+2.34x$
Emergency (days)	0.079	0.779	3.571	**0.060·**	0.242	0.623	0.450	$y=59.314-0.395x$	$y=73.592$ -$1.438x$
Total biomass	108.915	**0.000***	0.000	0.990	0.158	0.692	0.469	$y=0.151+0.192x$	$y=0.152+0.208x$
Aboveground biomass	46.321	**0.000***	0.107	0.745	0.041	0.841	0.280	$y=0.08+0.089x$	$y=0.062+0.094x$
Belowground biomass	123.769	**0.000***	0.169	0.682	0.939	0.334	0.496	$y=0.07+0.136x$	$y=0.090+0.114x$
Aboveground Biomass/total biomass	5.166	**0.025***	0.317	0.574	0.544	0.462	0.040	$y=0.487-0.033x$	$y=0.461-0.017x$
Belowground biomass/total biomass	5.166	**0.025***	0.317	0.574	0.544	0.462	0.040	$y=0.513+0.033x$	$y=0.539+0.017x$
Aboveground biomass/belowground biomass	5.557	**0.020***	1.477	0.226	2.115	0.148	0.031	$y=1.056-0.127x$	$y=0.884-0.030x$

Bold values indicate significant results

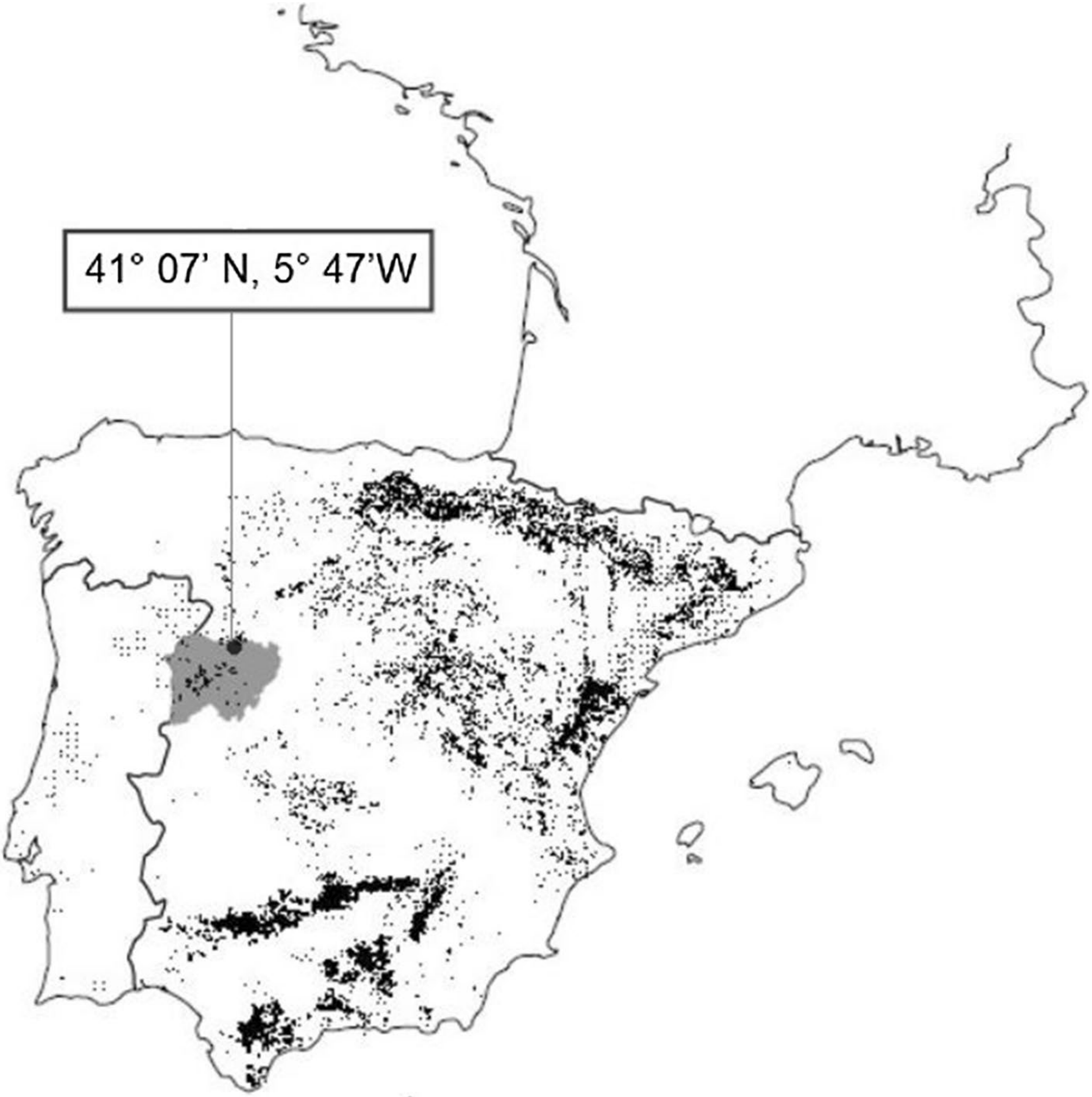


Fig. 1. Geographical location of the study area and distribution of *Quercus faginea* in the Iberian Peninsula. *Source* GBIF (2019)

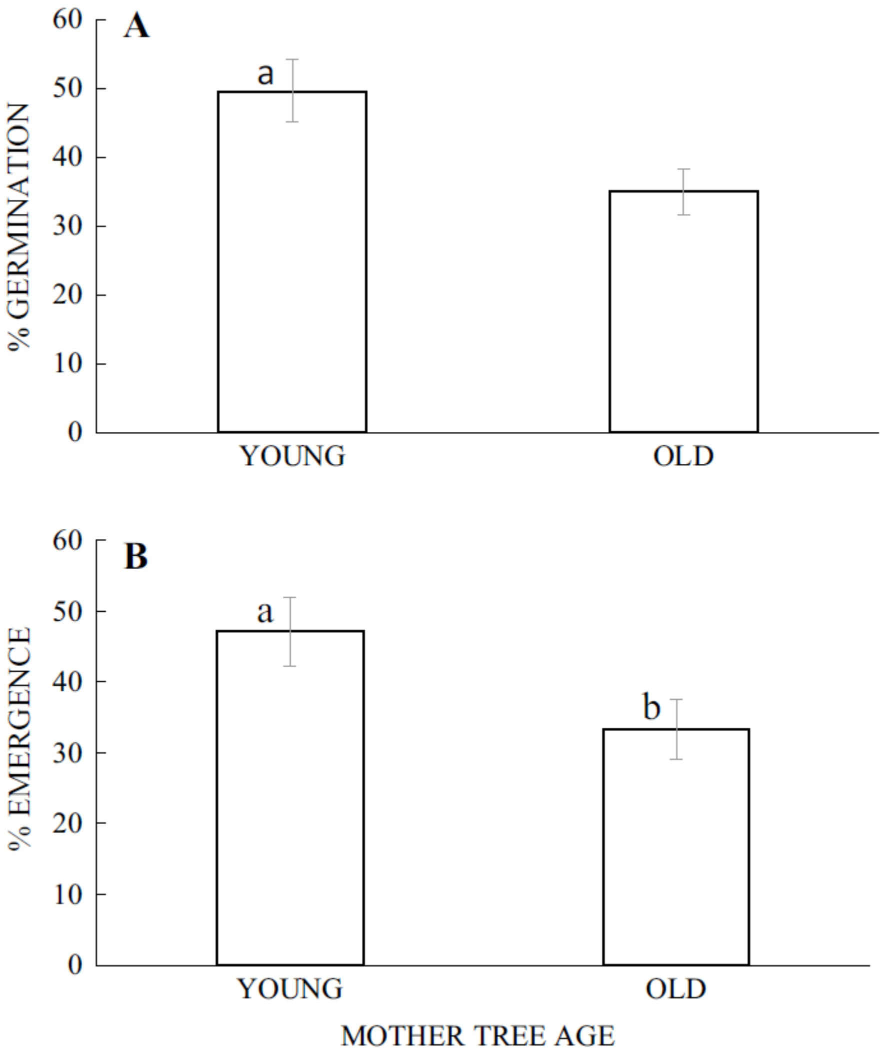


Fig. 2 Germination (**a**) and emergence (**b**) final percentage (mean value ± ES; $n = 8$) obtained for the young and old mother tree groups of *Q. faginea*. For each variable, different letters on the bars indicate significant differences with the Student's *t* test

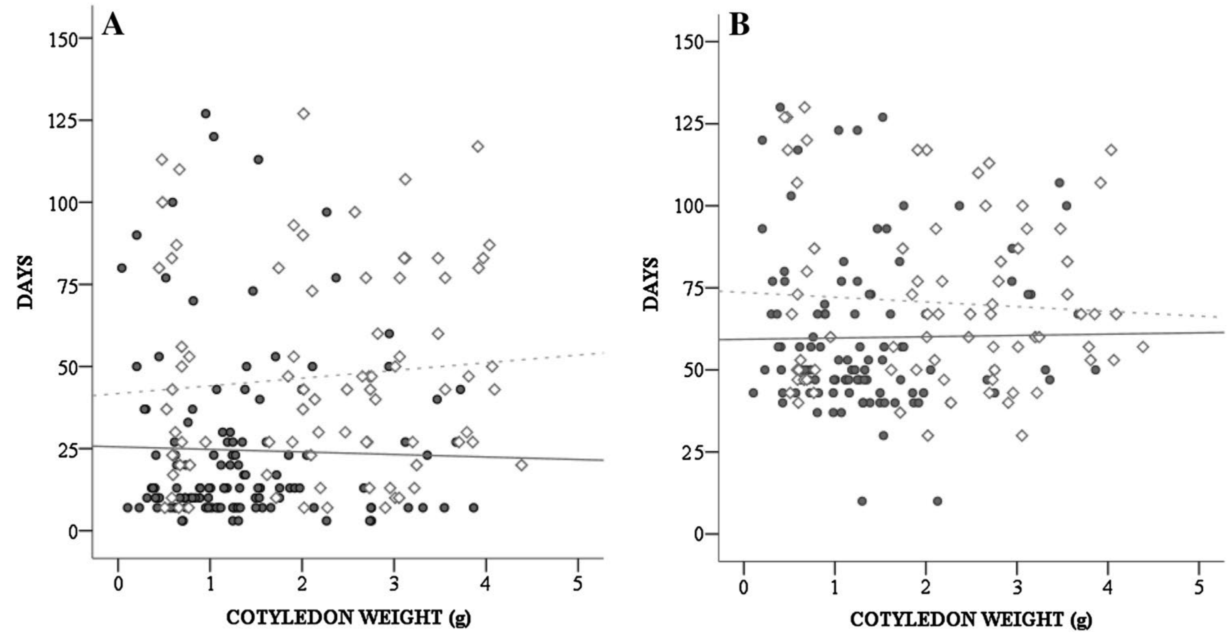


Fig. 3. Time elapsed (days) between seed sowing to germination (**a**) and seedling emergence (**b**), depending on the cotyledon dry weight and mother tree age. White rhombus for old tree acorns; black circle for young tree acorns; dotted line for old tree acorns; continuous lines for young tree acorns.

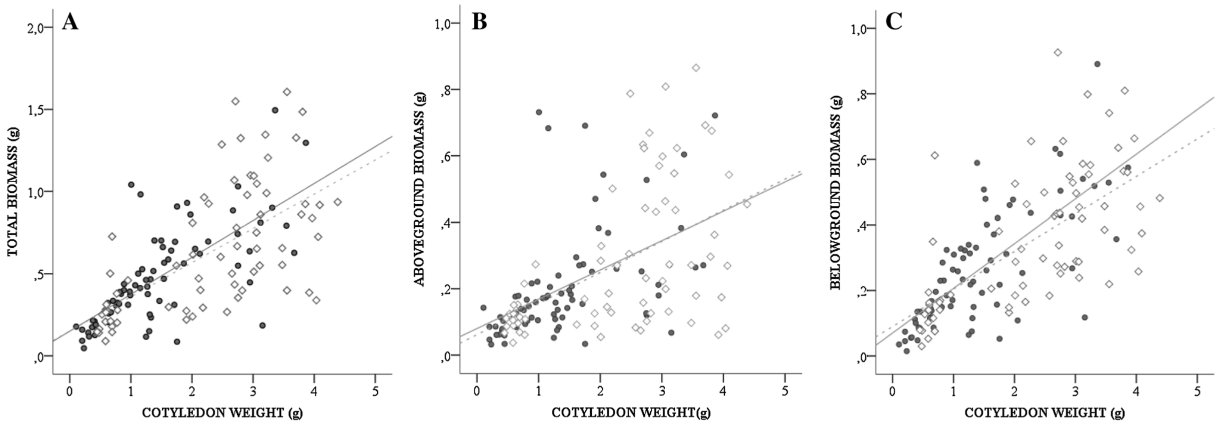


Fig. 4. Total biomass (g) (**a**) aboveground biomass (g) (**b**) and belowground biomass (g) (**c**) of the seedlings in relation to the cotyledon dry weight and the mother tree age. White rhombus for old tree acorns; black circle for young tree acorns; dotted line for old tree acorns; continuous lines for young tree acorns.

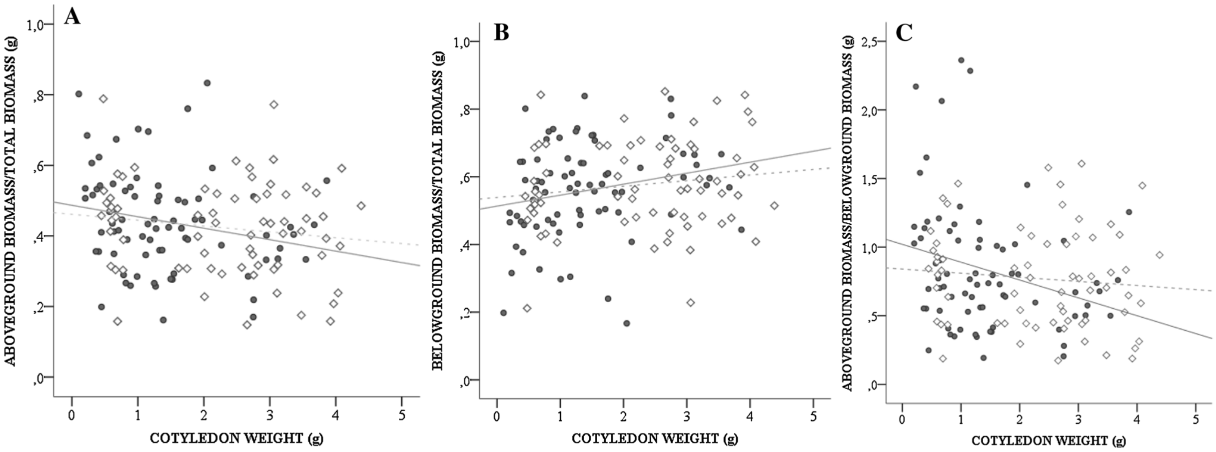


Fig. 5. Ratio aboveground biomass/total biomass (**a**), belowground biomass/total biomass (**b**) and aboveground biomass/belowground biomass (**c**) developed by the seedlings in relation to the cotyledon dry weight and the age of the mother tree. White rhombus for old tree acorns; black circle for young tree acorns; dotted line for old tree acorns; continuous lines for young tree acorns.

Appendix Legends

Appendix S1. Regression equations used to estimate both dry weights and cotyledon dry weights for each mother tree. The weights are expressed in grams. The values of N and R^2 of each regression are shown. All the regressions are significant.

Appendix S1

TREE	**REGRESSION LINE**	**R^2**	**N**	**TREE**	**REGRESSION LINE**	**R^2**	**N**
	Fresh weight - Cotyledon dry weight				Fresh weight - Dry weight		
Y1	y = 0,7027x - 0,2825	R^2 = 0,93609**	20	**Y1**	y = 0,7566x - 0,1678	R^2 = 0,92687**	20
Y2	y = 0,4935x + 0,0505	R^2 = 0,81826**	20	**Y2**	y = 0,5536x + 0,1586	R^2 = 0,82883**	20
Y3	y = 0,5856x - 0,1368	R^2 = 0,94588**	20	**Y3**	y = 0,7056x - 0,1063	R^2 = 0,94751**	20
Y4	y = 0,6572x - 0,3479	R^2 = 0,89538**	20	**Y4**	y = 0,7876x - 0,3087	R^2 = 0,93308**	20
Y5	y = 0,5726x - 0,041	R^2 = 0,93551**	20	**Y5**	y = 0,6346x + 0,0998	R^2 = 0,89618**	20
Y6	y = 0,5945x - 0,2127	R^2 = 0,96116**	19	**Y6**	y = 0,7671x - 0,2432	R^2 = 0,98834**	19
Y7	y = 0,5489x - 0,0456	R^2 = 0,9632**	19	**Y7**	y = 0,7278x - 0,1142	R^2 = 0,9847**	19
Y8	y = 0,5293x + 0,153	R^2 = 0,72182**	20	**Y8**	y = 0,7503x - 0,2259	R^2 = 0,87742**	20
O1	y = 0,6335x - 0,1321	R^2 = 0,87634**	20	**O1**	y = 0,7837x - 0,1523	R^2 = 0,86976**	20
O2	y = 0,6305x - 0,235	R^2 = 0,79586**	20	**O2**	y = 0,7555x - 0,1919	R^2 = 0,87717**	20
O3	y = 0,4677x + 0,3044	R^2 = 0,79118**	20	**O3**	y = 0,7386x - 0,1147	R^2 = 0,95219**	20
O4	y = 0,4784x + 0,2965	R^2 = 0,88796**	20	**O4**	y = 0,7063x - 0,0374	R^2 = 0,96419**	20
O5	y = 0,6129x - 0,3591	R^2 = 0,78059**	20	**O5**	y = 0,7893x - 0,4415	R^2 = 0,92966**	20

O6	$y = 0,5625x + 0,1135$	$R^2 = 0,90076$**	20	**O6**	$y = 0,7114x + 0,0258$	$R^2 = 0,93899$**	20
O7	$y = 0,6131x - 0,0974$	$R^2 = 0,87$**	20	**O7**	$y = 0,8078x - 0,3469$	$R^2 = 0,94394$**	20
O8	$y = 0,5543x + 0,0237$	$R^2 = 0,81321$**	20	**O8**	$y = 0,7219x - 0,1345$	$R^2 = 0,87565$**	20